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Short Communication

Dominance of two genotypes of *Bordetella pertussis* during a period of increased pertussis activity in Alberta, Canada: January to August 2012

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SUMMARY

Objectives: The purpose of this study was to undertake an epidemiological analysis of an increase in *Bordetella pertussis* activity during the period January 1 to August 31, 2012 in Alberta, Canada. *B. pertussis* testing was done using an *IS*481 real-time PCR assay with PCR-positive and indeterminate specimens cultured and stored for further analysis.
Methods: Laboratory data were linked to Alberta Health (AH) cases that were reported in the Communicable Disease Reporting System (CDRS) to identify case isolates; exclusion criteria were used to avoid biases. Case isolates were analyzed at the National Microbiology Laboratory (NML) by pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST). Pertussis immunization data were extracted from the Alberta Provincial Immunization Repository (Imm/ARI) and linked to the pertussis cases.
Results: Using PFGE and MLST, 52 case isolates could be divided into two main sequence type groups: 41 cases belonged to the ST-1 group (ST-1 and the novel ST-19) and 11 cases belonged to the ST-2 group (ST-2 and the novel ST-20). Of the total cases genotyped ($N = 52$), 18 (34.6%) had a history of immunization, 28 (53.8%) were not immunized, and six (11.6%) had an unknown immunization history. Of the total non-immunized cases, 25/28 (89.2%) belonged to the ST-1 group. Furthermore, of the 41 ST-1 group cases, 25 were not immunized compared to only three of the ST-2 group cases ($p = 0.0004$, Fisher's exact test).
Conclusions: This study shows the dominance of two genotypes of *B. pertussis* in our jurisdiction and indicates less pertussis immunization in individuals infected with the ST-1 group.

1. Introduction

In the spring of 2012, there was an increase in the incidence of pertussis in Alberta, Canada (population 3.8 million, 2012), which rapidly became a concern to public health practitioners. Also of concern was a significant increase in the incidence in children aged <5 years. The previous 5-year average for the same time period was 89.4 cases, and in the South and Edmonton zones the number of cases was 3 and 5 times as high, respectively.[1] Various causes for this increase were postulated, including non-vaccination, waning immunity, the relatively high sensitivity of *Bordetella pertussis* molecular testing platforms, and potential primary vaccine failure. Although vaccination status and molecular typing of isolates has been discussed in prior studies, little has been done to directly link vaccine status to genotype. The objective of this study was to describe the predominant genotype strains of *B. pertussis*-positive cases in the winter, spring, and summer of 2012 in Alberta, Canada, and to link genotype to vaccination status.

2. Methods

The Provincial Laboratory of Public Health (ProvLab) undertook an IS*481* PCR for *B. pertussis* detection from respiratory specimens as per routine, followed by culture confirmation of PCR-positive

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specimens, as described previously.[1,2] The selection of *B. pertussis* isolates for genotyping was as follows. Viable *B. pertussis*-positive culture isolates and cases were first linked and then isolates from known household contacts of first cases were excluded. As part of the non-routine public health investigation, culture-confirmed case isolates were analyzed at the National Microbiology Laboratory (NML) by pulsed-field gel electrophoresis (PFGE) and genotyping.[3] A multilocus sequence typing (MLST) approach was carried out using the five gene targets of *ptx*S1, *prn*, *fim*3, *fha*B, and *ptx*P.[3]

Alberta uses the Public Health Agency of Canada case definition of pertussis.[1] Laboratory data were linked to an Alberta Health (AH) case dataset to identify pertussis cases as reported in the automated provincial Communicable Disease Reporting System (CDRS). Immunization status was also linked to case data using CDRS.[4] SAS 9.2 (SAS Institute Inc., Cary, NC, USA) was used for all data manipulation and analyses. *p*-Values of $\leq$0.05 were considered significant. BioNumerics 5.10 (Applied Maths, Austin, TX) was used to create dendrograms for comparison of PFGE patterns.

3. Results

There were 217 cases in total, with 142 laboratory confirmed cases and 75 not laboratory confirmed. For genotyping, exclusion criteria included known household contacts of cases (n = 39), cases without clinical isolates (n = 75), and cases without viable isolates (n = 51). There were 52 isolates from cases submitted to the NML for genotyping. Pertussis cases selected for genotyping were similar to cases not typed with regard to factors such as gender (28/52, 54% male), median age (6 years), completion of immunization status (11/52, 21% complete for age), and association with outbreaks (10/52, 19%). Cases linked to typed isolates were more likely to be <1 year of age (16/52, 30.8%) than cases where no *B. pertussis* genotyping was done (14/185, 8.5%; p = 0.0003). Cases linked to typed isolates were more likely to be hospitalized (11/52, 21.2%) than cases where no *B. pertussis* genotyping was done (12/165, 7.3%; p = 0.014).

Standardized NML PFGE profiles are noted in Figure 1; AB alpha descriptors were given to each apparent strain. The percentage relatedness or similarities were calculated based on analysis of these isolates; as the number of strains to be analyzed increases, the percentage relatedness of strains may change, as described previously.[3] There were five different cluster patterns, and 16 different profiles were detected in which four profiles (profiles A, A1, A3, and F) were represented by more than one isolate. Profiles A1 to A7 were either closely related or possibly related to profile A. Profiles B, C, D, and E, belonging to a second cluster, were closely related and different from the third cluster with designated patterns F and G. The remaining two profiles, H and I, are independent from each other. All the profiles within the cluster are closely related to each other, but they are different from the rest of the other cluster profiles (Figure 1).

MLST results are available in Table 1. Combining MLST and PFGE data produced the following findings. Isolates with ST-1 and ST-19 MLST profiles linked to PFGE profiles A and A1–A7 (Figure 1). Isolates with ST-2 and ST-20 profiles linked to PFGE profiles B–I. For later epidemiological analysis, these ST-2 and ST-20 isolates were combined as ST-2 group. Novel genotypes were identified: a single isolate with a profile A1 as *prn*18 (GenBank accession number **KJ433480**), and a single isolate with a profile A1 as *ptx*P20 (GenBank accession number **KJ433481**).

B. pertussis vaccination histories of all cases, laboratory confirmed cases, and cases linked to PFGE are shown in Table 2. Of the 52 pertussis cases genotyped, 11 (21%) had a complete series of pertussis immunizations, seven (13%) had a partial series of pertussis immunizations, 28 (54%) had a definitive history of no pertussis immunizations, and six (12%) could not remember an immunization history to pertussis and the immunization repository had no records, or they were lost to follow-up. Of the 41 ST-1 cases, 25 had a definitive history of no pertussis immunizations compared to only three of the ST-2 cases (p = 0.0004, Fisher's exact test).

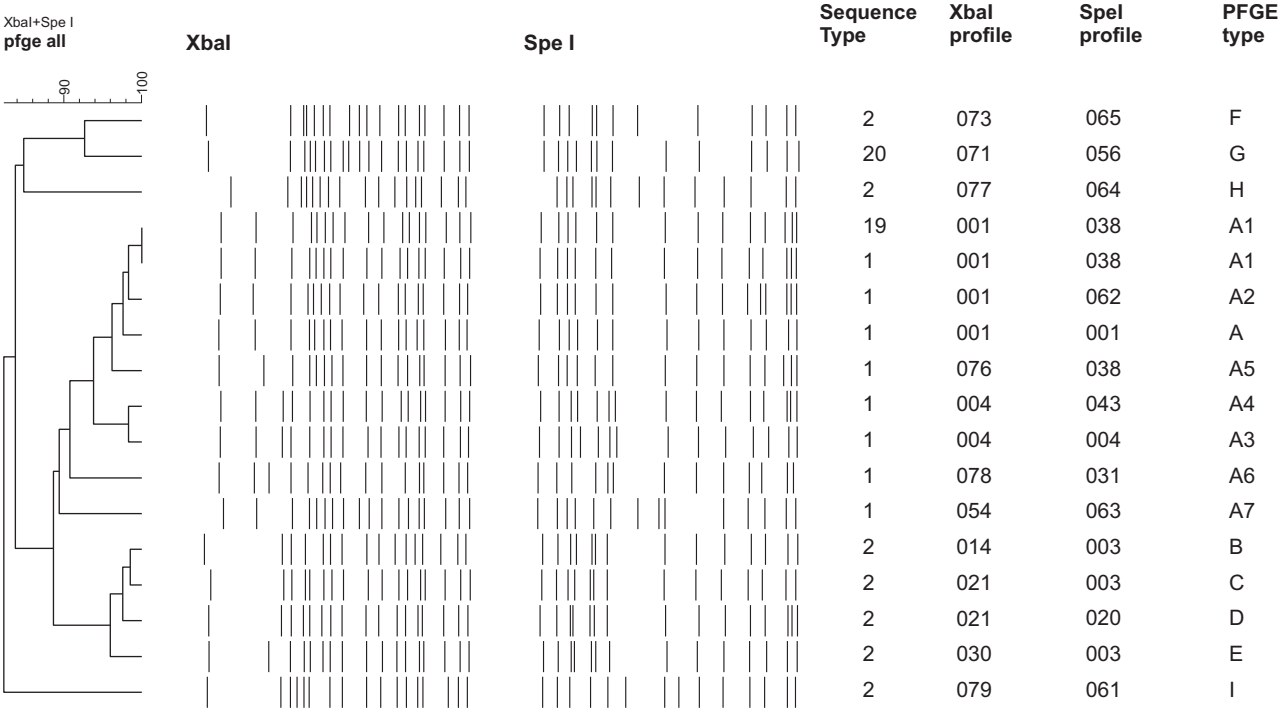

Figure 1. Dendrogram created in BioNumerics using *Xba*I and *Spe*I restricted DNA from 52 *Bordetella pertussis* strains isolated in Alberta, Canada in 2012.

Table 1
MLST types of *Bordetella pertussis* and specific loci descriptors

ST[a]	Number of strains	*ptx*S1	*prn*	*fim*3	*fha*B	*ptx*P
1	40	*ptx*S1A	*prn*2	*fim*3B	*fha*B1	*ptx*P3
2	10	*ptx*S1A	*prn*2	*fim*3A	*fha*B1	*ptx*P3
19	1	*ptx*S1A	*prn*18	*fim*3B	*fha*B1	*ptx*P3
20	1	*ptx*S1A	*prn*2	*fim*3A	*fha*B1	*ptx*P20

MLST, multilocus sequence typing.
[a] For later epidemiological analysis, ST-1 and ST-19 were defined as ST-1 group, and ST-2 and ST-20 as ST-2 group.

Table 2
Differences in immunization history between all cases, laboratory confirmed cases, and cases linked to genotyped isolates

	All cases	Laboratory confirmed only	Genotyped isolates
Completely immunized	53 (24%)	39 (27%)	11 (21%)
Partially immunized	31 (14%)	23 (16%)	7 (13%)
Not immunized	108 (50%)	65 (46%)	28 (54%)
Unknown	25 (12%)	15 (11%)	6 (12%)
Total	217	142	52

4. Discussion

The dominance of ST-1 group and ST-2 group MLST patterns of *B. pertussis* is similar to the patterns seen in Ontario, Canada,[3] with alleles similar to those seen recently in Washington State, USA.[5] *B. pertussis* genotype data should be interpreted in light of changes in vaccination strategies over the last 20 years in Alberta, which may have impacted vaccine coverage and efficacy, as well as circulating genotypes.[6–8] There have been several changes in pertussis vaccination in Alberta. 1n 1997, acellular pertussis vaccine replaced whole-cell vaccine, which had been used in Alberta since 1939. In September 2004, Adacel, a combined diphtheria, tetanus, and acellular pertussis vaccine, replaced a tetanus/diphtheria booster for grade 9 students for added protection. Furthermore, the infant formulation used for children aged 2 months to 7 years contains higher concentrations of *B. pertussis* antigens than the adult/adolescent vaccine.[1] As changes in vaccine composition have been postulated to impact on genotype, the genotyping in this study should be considered to be a snapshot of what was present in Alberta for a specific time period.[6]

Poor vaccine coverage in pertussis cases has been described previously in our region.[1] Although non-vaccination status was more likely to be associated with ST-1 group isolates and less with ST-2 group, the authors caution against over-interpretation of this point due to some potential biases, including biases in selection of isolates for typing from hospitalized patients and patients <1 year of age. In the future, analysis of trends in separate specific populations such as pediatric or hospitalized/community patients, or by degree of illness, may also provide a less biased analysis of the impact of variables on *B. pertussis* infection.[9]

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